Isolation of novel plant-beneficial soil bacteria to enhance legume crop productivity

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Abstract

Plant roots support the growth of a variety of soil microorganisms that have beneficial or detrimental effects on plant growth. As producers move to earlier seeding into cool wet soils, problems with pea and lentil seedling decay (damping off) and root rot are increasing. Attempts to control *Pythium* and *Fusarium spp.* with seed coatings containing the fungicides Captan or Thiram are being used however, the activity of these compounds is limited to approximately 2-3 weeks after planting. The objective of this project is to isolate microorganisms with the ability to be competitive in the rhizosphere of plants and biologically control root diseases of legumes.

Over six hundred isolates were obtained from the rhizosphere of chickpea, lentil and pea from commercial farms in Rosetown and North Battleford, Saskatchewan. Preliminary characterization of the isolates indicates that the collection consists of 16 actinomycetes, 4 yeasts and over 580 bacteria. Eighty percent of the isolates produced siderophores and the ability to inhibit the growth of *Rhizoctonia* and *Pythium* was observed in 11.6% and 7.5% of the isolates, respectively. Additional characterization of the isolates to be studied will be plant growth promotion, root colonization, growth at low temperature and identification.

Introduction

Pulse crop production has nearly doubled since 1997 from 3.16 to 5.96 million acres in 2000 and in Saskatchewan pulses were valued at $900 million in 2000. Increased production of pulse crops, particularly if rotations are shortened, promotes pulse diseases. Fungal diseases cause significant economic losses and are most evident above ground, however seedling decay (damping off) and root rot diseases are also important. Seedling diseases and root rot diseases impact on crop stand, root mass, root nodule formation, nitrogen fixation and yield. Damping-off and root rot are caused by *Fusarium solani*, *Pythium* spp and *Rhizoctonia solani,* all soil-borne pathogens commonly found in soil previously seeded to pulses. The factors contributing to seedling damping off and root rot of pulses include the following: 1. Early seeding, 2. High soil moisture, 3. Cool soil temperatures, 4. Previous crops, 5. Seed quality and 6. Lack of varietal disease resistance. Factors 1-3 are difficult for producers to control since the window for spring seeding is narrow and the growing season is short in the Northern grain belt. To reduce the incidence of root rot, seeding of pea should occur once in 5 years on the same land. Cereal crops are best rotated with pea, and dry bean, fababean and alfalfa should not be included in the rotation (Alberta Pulse Growers, 1993). Seed quality and the level of pathogens associated with the seed can be determined prior to seeding to ensure diseases are not being introduced. Varietal resistance to the root rot pathogens is not available. Chemical control of seedling diseases provides protection for 2-3 weeks after planting, but is less effective for control of *Rhizoctonia* spp.

Many complex interactions occur between plants and soil microorganisms in the rhizosphere, the dynamic zone in soil under the influence of plant roots, and include plant-beneficial, -neutral and -detrimental (pathogenic) interactions. The rhizosphere extends several centimeters from the root (Darrah 1993) and root exudates including organic acids, sugars, amino acids and minerals stimulate the soil micro-flora to colonize the rhizosphere and increase by as much as 1000 times the population observed in non-rhizosphere soil (Bowen and Rovira 1976, Jones 1998). Plant growth promoting rhizobacteria (PGPR) colonize the developing root and enhance plant growth by production of phytohormones, suppression of pathogenic microorganisms by production of antifungal metabolites, production of siderophores that chelate essential metabolites and induce resistance in plants to pathogens. In contrast to chemical control for protection, PGPR provide protection throughout the life of the plant.

The objectives of this study are to isolate PGPR suitable for control of fungal pathogens and plant growth promotion of pulses that:

- are adapted to local conditions
- colonize the rhizosphere of pulses
- produce anti-fungal metabolites that suppress *Fusarium, Pythium* and *Rhizoctonia*
- are compatible with *Rhizobium* and seed-applied fungicides
- grow at low temperature
- are amenable to commercial formulations

Material and Methods

Isolation of rhizosphere-colonizing microorganisms from pulse crops

Chickpea cv Stanford, lentil cv Crimson and pea cv Grande roots with adhering soil were collected in early July 2000 from 4 fields near Rosetown and 2 near North Battleford, Saskatchewan. Samples of root and adhering soil were added to 0.1M $MgSO_4$ buffer, shaken vigorously on a vortex mixer for 5 min. and diluted. Aliquots from the appropriate dilutions were spread onto one-tenth strength tryptic soy agar and Kings B dilute medium and incubated for 72 hours at 22°C. Over 600 hundred colonies were selected based on colony morphology, size, margin, shape and colour. All isolates were purified and frozen at –80°C in 20% glycerol. For experiments described below, isolates were thawed and an aliquot was transferred into half-strength tryptic soy broth and placed on a rotary shaker for 48h.

Root elongation assay

Surface-sterilized lentil seed cv Indian Head was inoculated with a 100-fold dilution of a 48 hour-old culture (about 10^6 microorganisms/mL). Lentil root elongation was determined using a sterile plant growth pouch system (Lifshitz et al. 1987). Seven seeds (inoculated or non-inoculated) were placed in a sterile growth pouch containing 10 mL of sterile Hoaglands N-free nutrient solution. There were seven growth pouches per treatment. Growth pouches were wrapped in plastic film to reduce evaporation, packaged tightly together to promote vertical root growth and placed in a growth chamber with day/night temperatures of 20/17°C and a 14-hour photoperiod. Root length was determined 6 days after planting.

Production of antifungal metabolites

An *in vitro* assay was used to examine inhibition of growth of *Pythium* and *Rhizoctonia* by the rhizosphere isolates. A 6-mm plug of *Pythium* or *Rhizoctonia* was placed in the center of one-fifth strength potato dextrose agar medium (PDA). Growth of the fungus was challenged by inoculating a single streak (2 cm) of the soil isolate on the PDA plate 3 cm from the fungal plug. Suppression of fungal growth was measured at 48 and 72 hours of incubation. The data presented below were collected at 72 hours.

Production of siderophores

The chrome Azurol S (CAS)method (Schwyn and Neiland (1987) was used to determine siderophore production by the soil-rhizosphere isolates. The test was carried out in 96-well plates. An orange-yellow colour indicated siderophore production and a blue colour indicated no siderophore production. The rhizosphere isolates were grown for 48 hours in rhizosphere medium (RSM) (Buyer et al. 1989). This low iron-containing medium encourages the production of siderophores and has sufficient carbon to promote growth. Aliquots of the isolates were transferred into the wells containing fresh RSM and the colour indicator CAS. Colour change was recorded after 48 hours.

Results and Discussion

Isolation of rhizosphere-colonizing microorganisms from pulse crops

Approximately 1 to 2 X 10^7 microorganisms/g fresh weight of root from chickpea, lentil and pea and adhered soil were observed on one-tenth strength TSA and about 10-fold less on the King's B agar. From the 10^{-4} dilution plates 652 colonies were selected for further purification. From this initial purification step 600 isolates were regenerated and identified as 580 bacteria, 16 actinomycetes and 4 yeasts.

Root elongation assay

The ability of the rhizosphere isolates to promote lentil root elongation was examined in plant growth pouches. A preliminary experiment was carried out to demonstrate the ability of PGPR to promote root elongation of lentil. Strain 31-12 was reported to promote root elongation of canola (Kloepper et al. 1988) and was included in this experiment. All of the isolates significantly promoted root elongation of lentil (Figure 1). Root length of lentil inoculated with 31-12 was up to 2.6 cm longer than the non-inoculated seedlings (Figure 1). Root elongation can be advantageous if the seedling is seeking moisture or nutrients lower in the soil profile. Studies are underway to screen all of the rhizosphere isolates for root elongation.

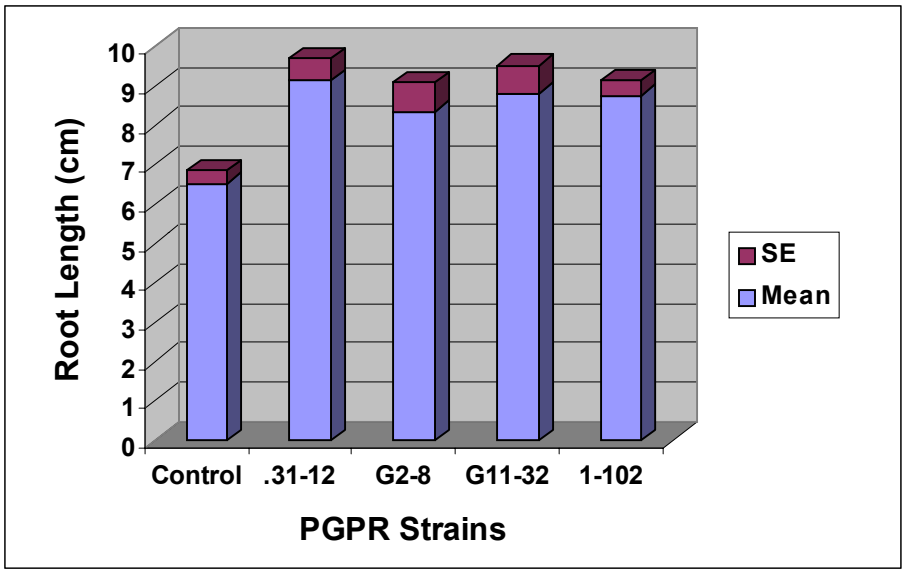

Figure 1. Effect of PGPR on root elongation of lentil cv Indian Head.

Production of antifungal metabolites

The ability of the rhizosphere isolates to suppress the growth of *Pythium, Rhizoctonia* and *Fusarium*, phytopathogens responsible for seedling damping off and root rot, was examined using an *in vitro* assay. Of the 580 bacterial isolates tested, 11.6% suppressed the growth of *Rhizoctonia* and 7.5% suppressed the growth of *Pythium* (Figure 2). Studies are underway to

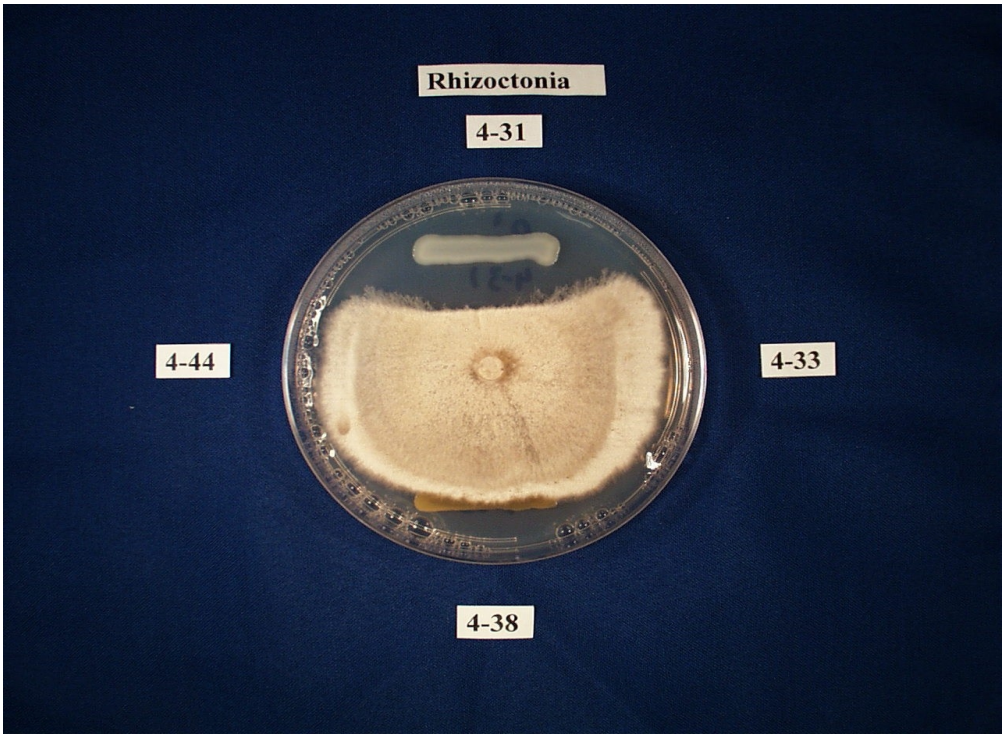

Figure 2. *In vitro* assay for anti-fungal metabolite production.

determine the percent suppression of *Fusarium* by the rhizosphere isolates. Suppression of fungal growth on agar plates is a rapid initial test to select biological control agents. Greenhouse and field studies are used to further characterize and obtain efficacy data on the bio-fungicidal activity of the rhizosphere isolates.

Production of siderophores

Of the 580 isolates 80% were capable of producing siderophores. The low iron-containing medium used encourages the production of siderophores and has sufficient carbon to promote growth. The ability to produce siderophores and chelate nutrients essential for proliferation of pathogens is an indirect mechanism by which soil microorganisms promote plant growth.

Future studies

Future studies that are planned include:

- ability to grow at low temperature (5-15 ^{O}C)
- ability to colonize roots
- ability to produce phytohormones
- ability to decrease ethylene production in plants
- ability to reduce soil-borne fungal pathogen diseases *in vivo*

From these 600 rhizosphere isolates the 10 most promising candidates will be selected for further study. Their ability to promote growth and reduce damping off and root rot in pot studies, their compatibility with *Rhizobium* inoculants and seed-applied fungicides and finally their ability to promote growth of pulses in the field will be assessed. The potential to incorporate these into sustainable management practices will require testing the survival of the rhizosphere isolates in commercial formulations.

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